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Antibiotic Susceptibility of Skin Swab *Staphylococcus* Isolates From Patients With Diarrhea-Predominant Irritable Bowel Syndrome Treated With Repeat Courses of Rifaximin Show No Evidence of Resistance

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INTRODUCTION

- Compared with healthy individuals, patients with irritable bowel syndrome (IBS) have altered gut microbiota,[1,2] suggesting that the gut microbiota may be an appropriate target for the development of treatments for diarrhea-predominant IBS (IBS-D)
- Rifaximin is an oral, nonsystemic antibiotic approved in May 2015 for IBS-D in adults; the efficacy and safety of a single 2-week course of rifaximin 550 mg 3 times daily (TID) for improving IBS symptoms was demonstrated in two phase 3, randomized, placebo-controlled trials (TARGET 1 and 2)[3]
- In a phase 3 trial of rifaximin repeat treatment (TARGET 3), patients who initially responded to rifaximin and had IBS symptom recurrence were randomly assigned to receive 2 courses of rifaximin or placebo; compared with patients receiving placebo, a significantly larger percentage of rifaximin-treated patients had improvement in a composite endpoint of IBS-related abdominal pain and stool consistency[4]
- Rifaximin inhibits bacterial RNA synthesis[5] and has in vitro bactericidal activity against aerobic and anaerobic bacteria, including *Staphylococcus aureus*[6,7]
- Although rifaximin may not alter the overall nonpathogenic bacterial load of the gut microbiota,[8,9] the potential risk of antibiotic resistance, including cross-resistance, remains a potential clinical concern

AIM

- To analyze antibiotic susceptibility of *Staphylococcus* strains cultured from skin-swab samples in patients with IBS-D who received single or repeat treatment with rifaximin

METHODS

Study Design and Patient Population

- Patients were eligible for the exploratory study if they were enrolled at one of the sites participating in the substudy
  - Sites were selected based on expressed interest, responses to a feasibility survey, and staff experience with collecting skin-swab samples
- Eligible adults had a diagnosis of IBS-D (Rome III criteria) and inadequate relief of global IBS symptoms and IBS-related bloating during screening
  - Patients taking probiotics or taking rifaximin or any other antibiotic ≤14 days before providing written informed consent were excluded
- Patients received open-label (OL) rifaximin 550 mg TID for 2 weeks followed by a 4-week, treatment-free follow-up
  - Rifaximin responders met weekly response criteria for IBS-related abdominal pain (≥30% decrease from baseline in mean weekly pain score) and stool consistency (≥50% decrease from baseline in number of days/week with Bristol Stool Scale stool types 6 or 7) during ≥2 of the 4 weeks post-treatment
  - Nonresponders were withdrawn from the trial
- Responders were followed for up to 18 weeks during a treatment-free observation period
  - Patients experiencing symptom recurrence (loss of treatment response for weekly IBS-related abdominal pain or stool consistency for ≥3 weeks of a consecutive, rolling 4-week period during the observation phase) were randomly assigned (1:1) to receive 2 repeat double-blind (DB) courses, each course separated by 10 weeks, of rifaximin 550 mg or placebo TID for 2 weeks

Skin Swab Collection and Isolate Culture, Identification, and Susceptibility Testing

- Skin swabs were collected at 5 time points: (1) OL baseline; (2) end of 2-week treatment with OL rifaximin; (3) DB baseline; (4) end of the first repeat treatment with rifaximin or placebo for 2 weeks; and (5) at the end of the study
- Swab samples were collected from the perianus, both nostrils, both forearms, and the palms of the hands
- Cultures were analyzed at central labs and skin swabs were plated on standardized agar mixture and incubated for 24 or 48 hours
- A latex agglutination test was performed to confirm *S aureus*, and molecular identification systems were used as needed to identify non–beta-hemolytic colonies or colonies found to be negative via other types of tests; if definitive identification was not possible, the isolate was reported using the characteristics identified (eg, catalase-negative *Staphylococcus*)
- Broth microdilution was used to determine the minimum inhibitory concentration (MIC) against 11 antibiotics: rifaximin, rifampin, ceftazidime, ceftriaxone, cephalothin, ciprofloxacin, imipenem, meropenem, piperacillin/tazobactam, trimethoprim/sulfamethoxazole, and vancomycin; tested MIC ranges and breakpoints were based on Clinical and Laboratory Standards Institute guidelines,[10] package inserts, or the published literature[11]

METHODS

- For each staphylococcal isolate, MIC data were compiled by visit and treatment (eg, OL rifaximin, DB rifaximin, DB placebo)
  - For comparisons between rifaximin and rifampin, the rifampin MIC value for categorizing resistance (ie, MIC ≥4 µg/mL) was extended to rifaximin

RESULTS

- Skin swabs were obtained for 115 patients during the OL phase; 31 of the 115 patients continued in the DB phase (rifaximin [n = 19]; placebo [n=12])
- 1381 isolates (18 strains) were identified; the most prevalent was *Staphylococcus epidermis* (54.2%; Table 1)

Table 1. Primary Staphylococcal Skin Isolates

Staphylococcus Species[a]	Isolates, n (%)
All *Staphylococcus* species	1381 (100.0)
Staphylococcus epidermidis	749 (54.2)
Staphylococcus hominis	238 (17.2)
Staphylococcus haemolyticus	113 (8.2)
Staphylococcus aureus	71 (5.1)
Staphylococcus capitis	60 (4.3)
Staphylococcus lugdunensis	49 (3.5)
Staphylococcus warneri	41 (3.0)
Staphylococcus simulans	17 (1.2)
Staphylococcus saprophyticus	14 (1.0)

[a]Isolates representing <1% of total: *Staphylococcus cohnii*, *Staphylococcus pasteuri*, *Staphylococcus pettenkoferi*, *Staphylococcus caprae*, *Staphylococcus intermedius*, *Staphylococcus carnosus*, *Staphylococcus xylosus*, *Staphylococcus schleiferi*, and coagulase-negative *Staphylococcus*.

Rifaximin and Rifampin Susceptibility Profile

- On Day 1 of the OL phase, 373 isolates were cultured; the number decreased during ensuing weeks (Table 2)
  - For rifaximin and rifampin, MIC_{50} values were similar; MIC_{90} was highest at Week 2 (end of OL rifaximin), then markedly lower at subsequent treatment-free visits, with a rapid return to the OL baseline value
- On Day 1 of the DB phase, 113 isolates were obtained; total number of isolates varied during this phase (Table 3)
  - Rifaximin and rifampin MIC_{50} values remained low for rifaximin and placebo groups; transient changes in rifaximin and rifampin MIC_{90} were observed in the DB rifaximin group but not in the DB placebo group, with a recovery close to the MIC_{90} baseline level by the end of the study

Table 2. In Vitro Activity of Rifaximin and Rifampin Against *Staphylococcus* Isolates Obtained During the OL Phase

		Rifaximin, µg/mL			Rifampin, µg/mL		
Time Point[a] (Patients)	Isolates, n	MIC Range	MIC_{50}	MIC_{90}	MIC Range	MIC_{50}	MIC_{90}
Day 1 (n = 113)	373	≤0.001–0.25	0.015	0.03	≤0.015–0.12	≤0.015	≤0.015
Week 2; EOT (n = 108)	336	≤0.001–128	0.015	32	≤0.015–>32	≤0.015	16
Week 7–10 (n = 25)	73	≤0.001–64	0.015	2	≤0.015–>32	≤0.015	0.5
Week 11–14 (n = 62)	250	≤0.001–64	0.015	0.03	≤0.015–>32	≤0.015	≤0.015
Week 15–18 (n = 10)	48	≤0.001–64	0.015	0.03	≤0.015–16	≤0.015	≤0.015
Week 19–22 (n = 6)	21	0.002–0.03	0.015	0.03	≤0.015–≤0.015	≤0.015	≤0.015
Week ≥23 (n = 3)	14	0.004–0.06	0.015	0.03	≤0.015–≤0.015	≤0.015	≤0.015

[a]Follow-up periods varied; therefore, follow-up visits were grouped into 4-week periods to determine whether time affected the susceptibility of isolates to antibiotics.
EOT = end of treatment; MIC = minimum inhibitory concentration; OL = open label.

RESULTS

Table 3. In Vitro Activity of Rifaximin and Rifampin Against *Staphylococcus* Isolates Obtained During the DB Phase

		Rifaximin, µg/mL			Rifampin, µg/mL		
Time Point[a] (Patients)	Isolates, n	MIC Range	MIC_{50}	MIC_{90}	MIC Range	MIC_{50}	MIC_{90}
Double-blind rifaximin							
Day 1 (n = 18)	65	≤0.001–64	0.015	0.03	≤0.015–>32	≤0.015	≤0.015
Week 2; EOT (n = 18)	64	0.004–64	0.015	32	≤0.015–>32	≤0.015	16
Week 11–14 (n = 1)	5	0.008–64	0.06	64	≤0.015–>32	0.03	>32
Week 15–18 (n = 1)	3	0.015–0.5	0.015	0.5	≤0.015–0.25	≤0.015	0.25
Week 19–22 (n = 10)	43	0.008–0.32	0.015	0.5	≤0.015–>32	≤0.015	0.12
Week ≥23 (n = 6)	28	0.004–64	0.015	0.06	≤0.015–>32	≤0.015	≤0.015
Double-blind placebo							
Day 1 (n = 12)	48	≤0.001–64	0.015	0.03	≤0.015–8	≤0.015	≤0.015
Week 2; EOT (n = 12)	63	≤0.001–64	0.015	0.03	≤0.015–>32	≤0.015	≤0.015
Week 15–18 (n = 1)	4	0.008–0.03	0.03	0.03	≤0.015–≤0.015	≤0.015	≤0.015
Week 19–22 (n = 5)	27	0.004–0.03	0.015	0.03	≤0.015–≤0.015	≤0.015	≤0.015
Week ≥23 (n = 6)	29	0.008–0.06	0.015	0.03	≤0.015–0.03	≤0.015	≤0.015

[a]Follow-up periods varied; therefore, follow-up visits were grouped into 4-week periods to determine whether time affected the susceptibility of isolates to antibiotics. Values shown are from only the weeks in which isolates were obtained.
DB = double blind; EOT = end of treatment; MIC = minimum inhibitory concentration.

Resistance Patterns

- No rifaximin- or rifampin-resistant *S aureus* isolates were identified during the study (either OL or DB phase)
- In the OL phase, none of the 373 isolates assessed at OL baseline were resistant to rifampin or rifaximin (Table 4)
  - During this phase, the most frequent site for rifaximin- and rifampin-resistant isolates was the perianus
  - Resistance patterns were generally similar for rifaximin and rifampin; no rifaximin- or rifampin-resistant isolates were identified after Weeks 15–18
- In the DB phase, the perianus was the most frequent site from which *Staphylococcus* isolates were obtained (Table 5)
  - Few rifaximin- or rifampin-resistant isolates were cultured from placebo-treated patients; all were collected from the perianus
  - No rifaximin- or rifampin-resistant isolates were identified after Weeks 15–18 in the placebo group
  - Among rifaximin-treated patients, the largest number of rifaximin- and rifampin-resistant isolates were observed at Week 2, which were all collected from the perianus

Table 4. Rifaximin- and Rifampin-Resistant *Staphylococcus* Isolates Obtained During the OL Phase

		Location of Antibiotic-Resistant Isolates									
		Rifaximin[a]					Rifampin				
Time Point[b] (Patients)	Isolates, n	Arms[c]	Nostrils	Palms	Perianus	Total	Arms[c]	Nostrils	Palms	Perianus	Total
Day 1 (n = 113)	373	0	0	0	0	0	0	0	0	0	0
Week 2; EOT (n = 108)	336	3	0	4	35	42	3	0	4	32	39
Week 7–10 (n = 25)	73	1	0	1	5	7	1	0	1	5	7
Week 11–14 (n = 62)	250	1	1	2	5	9	1	1	1	4	7
Week 15–18 (n = 10)	48	1	0	0	1	2	1	0	0	1	2
Week 19–22 (n = 6)	21	0	0	0	0	0	0	0	0	0	0
Week ≥23 (n = 3)	14	0	0	0	0	0	0	0	0	0	0

[a]To compare sensitivity of isolates to rifaximin and rifampin, the CLSI-established MIC breakpoint for rifampin (ie, resistance at MIC ≥4 µg/mL) was applied to rifaximin.
[b]Follow-up periods varied; therefore, follow-up visits were grouped into 4-week periods to determine whether time affected the susceptibility of isolates to antibiotics.
[c]Data for 2 forearms pooled.
CLSI = Clinical and Laboratory Standards Institute; EOT = end of treatment; MIC = minimum inhibitory concentration; OL = open label.

RESULTS

Table 5. Rifaximin- and Rifampin-Resistant *Staphylococcus* Isolates Obtained During the DB Phase

		Location of Antibiotic-Resistant Isolates									
		Rifaximin[a]					Rifampin				
Time Point[b] (Patients)	Isolates, n	Arms[c]	Nostrils	Palms	Perianus	Total	Arms[c]	Nostrils	Palms	Perianus	Total
Double-blind rifaximin											
Day 1 (n = 18)	65	1	0	0	1	2	1	0	0	1	1
Week 2; EOT (n = 18)	64	0	0	0	12	12	0	0	0	11	11
Week 11–14 (n = 1)	5	0	0	0	2	2	0	0	0	2	2
Week 15–18 (n = 1)	3	0	0	0	0	0	0	0	0	0	0
Week 19–22 (n = 10)	43	0	0	0	4	4	0	0	0	4	4
Week ≥23 (n = 6)	28	0	0	0	2	2	0	0	0	2	2
Double-blind placebo											
Day 1 (n = 12)	48	0	0	0	1	1	0	0	0	1	1
Week 2; EOT (n = 12)	63	0	0	0	2	2	0	0	0	2	2
Week 11–14 (n = 0)	0	0	0	0	0	0	0	0	0	0	0
Week 15–18 (n = 1)	4	0	0	0	0	0	0	0	0	0	0
Week 19–22 (n = 5)	27	0	0	0	0	0	0	0	0	0	0
Week ≥23 (n = 6)	29	0	0	0	0	0	0	0	0	0	0

[a]To compare sensitivity of isolates to rifaximin and rifampin, the CLSI-established MIC breakpoint for rifampin (ie, resistance at MIC ≥4 µg/mL) was applied to rifaximin.
[b]Follow-up periods varied; therefore, follow-up visits were grouped into 4-week periods to determine whether time affected the susceptibility of isolates to antibiotics. Values shown are only from the weeks in which isolates were obtained.
[c]Data for 2 forearms pooled.
CLSI = Clinical and Laboratory Standards Institute; DB = double blind; EOT = end of treatment; MIC = minimum inhibitory concentration.

MIC Analyses for Other Antibiotics Tested

- During the OL and DB phases, and regardless of treatment (OL rifaximin, DB rifaximin, DB placebo), MIC values were low among the 9 other antibiotics tested; any changes in MIC values were minimal and did not indicate the development of resistance after rifaximin exposure (data not shown)

CONCLUSIONS

- **Up to three 2-week courses of nonsystemic rifaximin in patients with IBS-D did not result in clinically significant antibiotic resistance in *Staphylococcus* skin isolates**
  - **Transient changes in MIC values, when observed for rifaximin or rifampin, returned to baseline levels during both the OL and the DB phases and were not considered clinically meaningful; neither rifaximin nor rifampin are first-line treatments for staphylococcal infections in clinical practice**
  - **Rifaximin did not appear to increase the risk for pathogenic bacteria, including *S aureus***
  - **There was no evidence of cross-resistance with other antibiotics tested**

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